Fig.1.

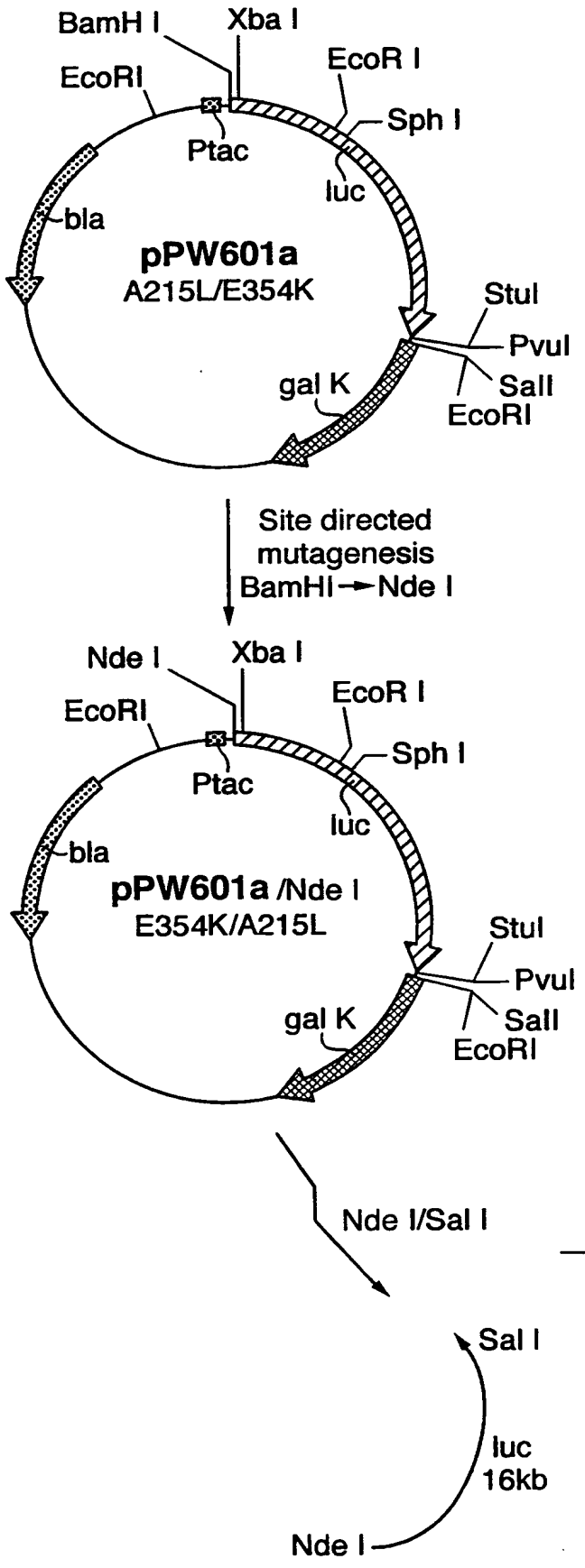
BamH I
Xba I
EcoR I
EcoRI
Sph I
Ptac
luc
bla
pPW601a
A215L/E354K
StuI
PvuI
SalI
gal K
EcoRI
Site directed
mutagenesis
BamHI→Nde I
Nde I
Xba I
EcoR I
EcoRI
Sph I
Ptac
luc
bla
pPW601a /Nde I
E354K/A215L
StuI
PvuI
SalI
gal K
EcoRI
Nde I/Sal I
Sal I
luc
16kb
Nde I

Fig.1 (Cont.)

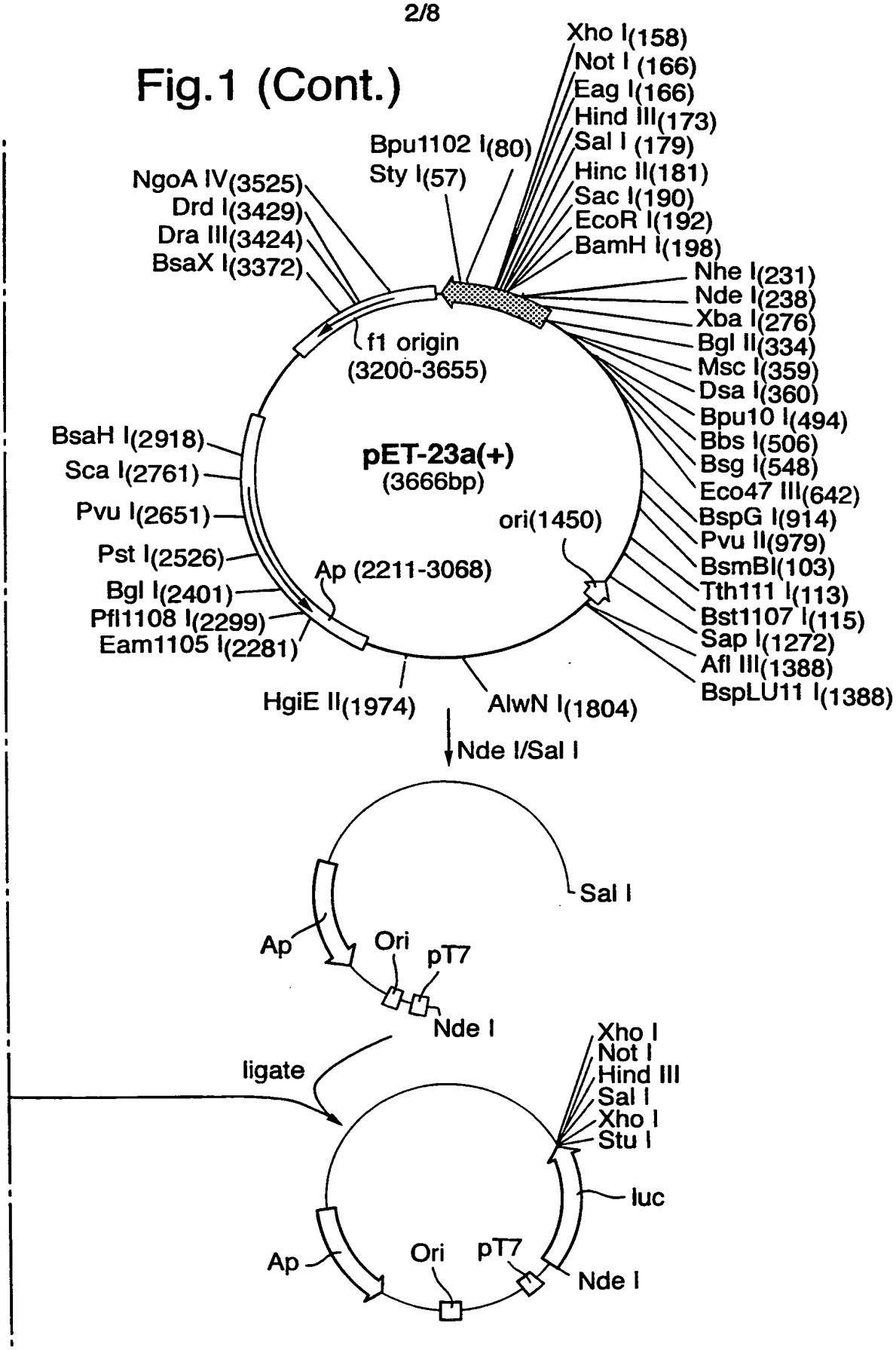
Xho I(158)
Not I (166)
Eag I(166)
Hind III(173)
Sal I (179)
Hinc II(181)
Sac I(190)
EcoR I(192)
BamH I(198)
Bpu1102 I(80)
Sty I(57)
NgoA IV(3525)
Drd I(3429)
Dra III(3424)
BsaX I(3372)
Nhe I(231)
Nde I(238)
Xba I(276)
Bgl II(334)
Msc I(359)
Dsa I(360)
Bpu10 I(494)
Bbs I(506)
Bsg I(548)
Eco47 III(642)
BspG I(914)
Pvu II(979)
BsmBI(103)
Tth111 I(113)
Bst1107 I(115)
Sap I(1272)
Afl III(1388)
BspLU11 I(1388)
f1 origin
(3200-3655)
pET-23a(+)
(3666bp)
ori(1450)
Ap (2211-3068)
BsaH I(2918)
Sca I(2761)
Pvu I(2651)
Pst I(2526)
Bgl I(2401)
Pfl1108 I(2299)
Eam1105 I(2281)
HgiE II(1974)
AlwN I(1804)
Nde I/Sal I
Sal I
Ap
Ori
pT7
Nde I
ligate
Xho I
Not I
Hind III
Sal I
Xho I
Stu I
luc
Ap
Ori
pT7
Nde I

Fig.2.

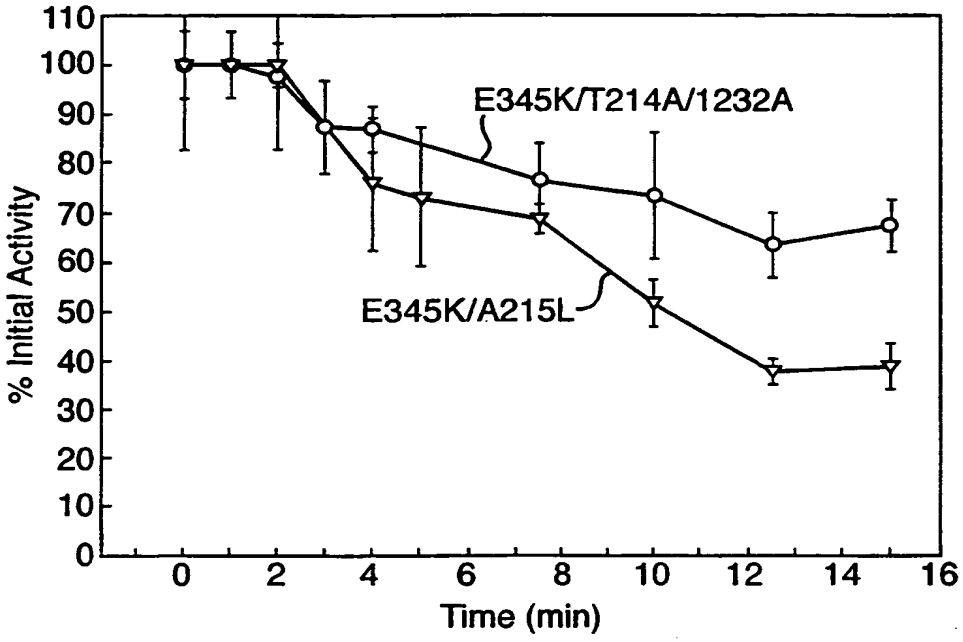
E345K/T214A/1232A
E345K/A215L
% Initial Activity
Time (min)
0
10
20
30
40
50
60
70
80
90
100
110
0
2
4
6
8
10
12
14
16

Fig.4.

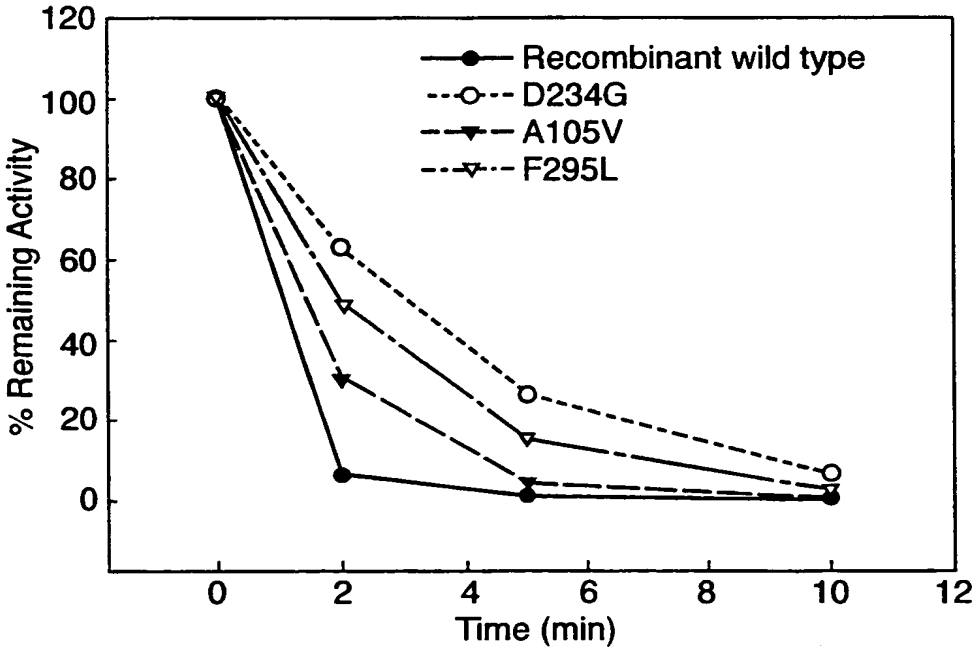
Recombinant wild type
D234G
A105V
F295L
% Remaining Activity
Time (min)
0
20
40
60
80
100
120
0
2
4
6
8
10
12

Fig.3a.

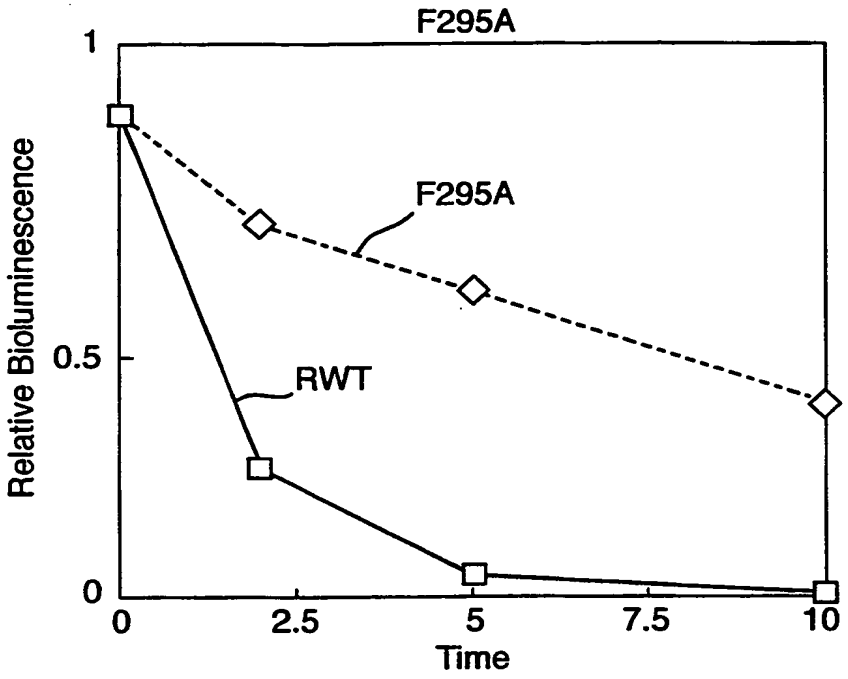
F295A
1
0.5
0
Relative Bioluminescence
F295A
RWT
0
2.5
5
7.5
10
Time

Fig.3b.

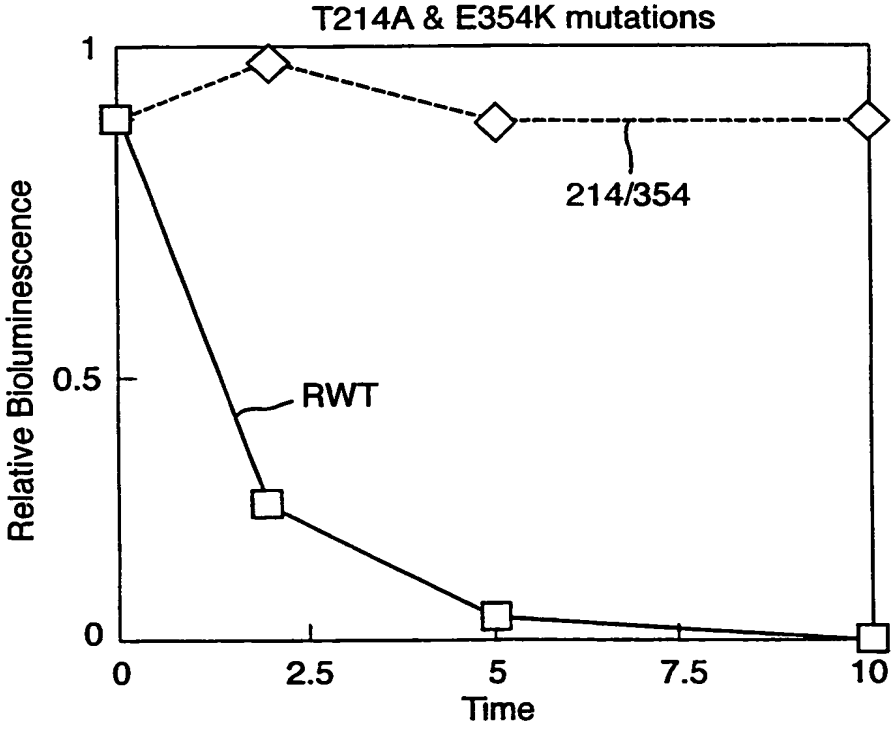
T214A & E354K mutations
1
0.5
0
Relative Bioluminescence
214/354
RWT
0
2.5
5
7.5
10
Time

Fig.3c.

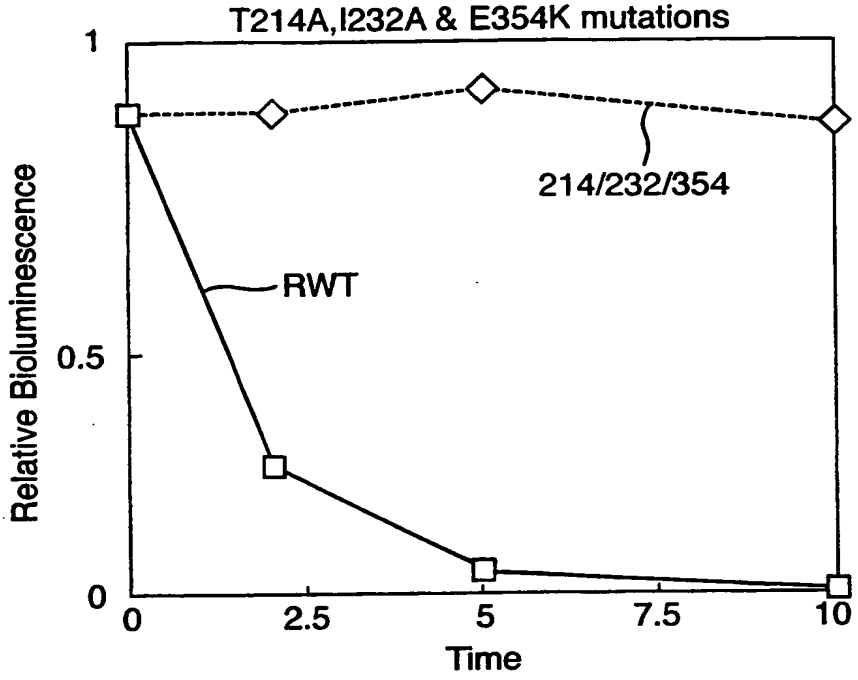
T214A,I232A & E354K mutations
Relative Bioluminescence
1
0.5
0
214/232/354
RWT
0
2.5
5
7.5
10
Time

Fig.3d.

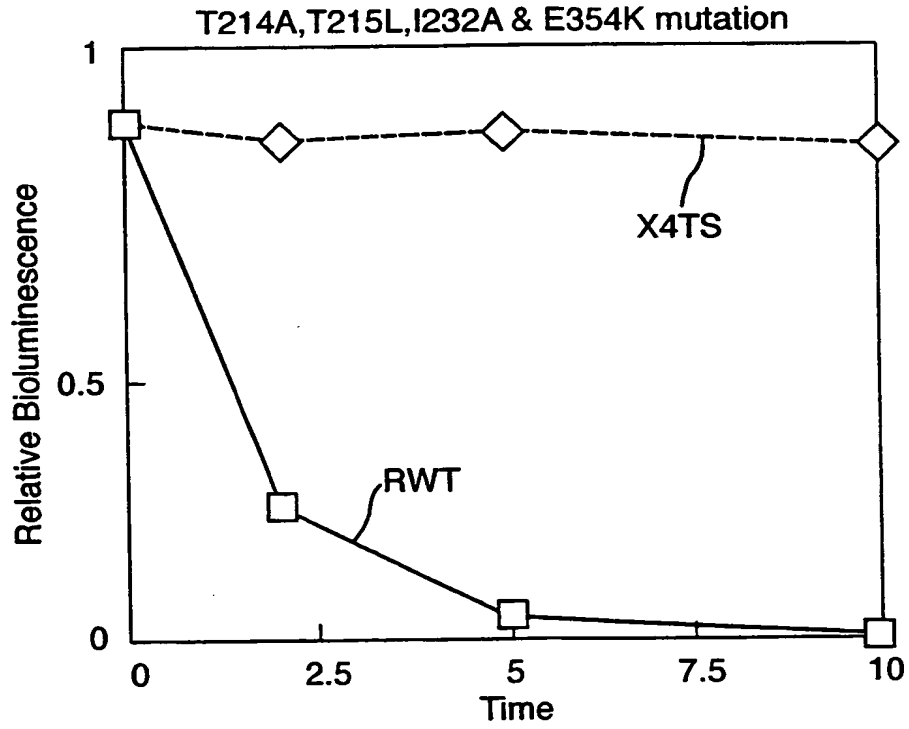
T214A,T215L,I232A & E354K mutation
Relative Bioluminescence
1
0.5
0
X4TS
RWT
0
2.5
5
7.5
10
Time

Fig.3e.

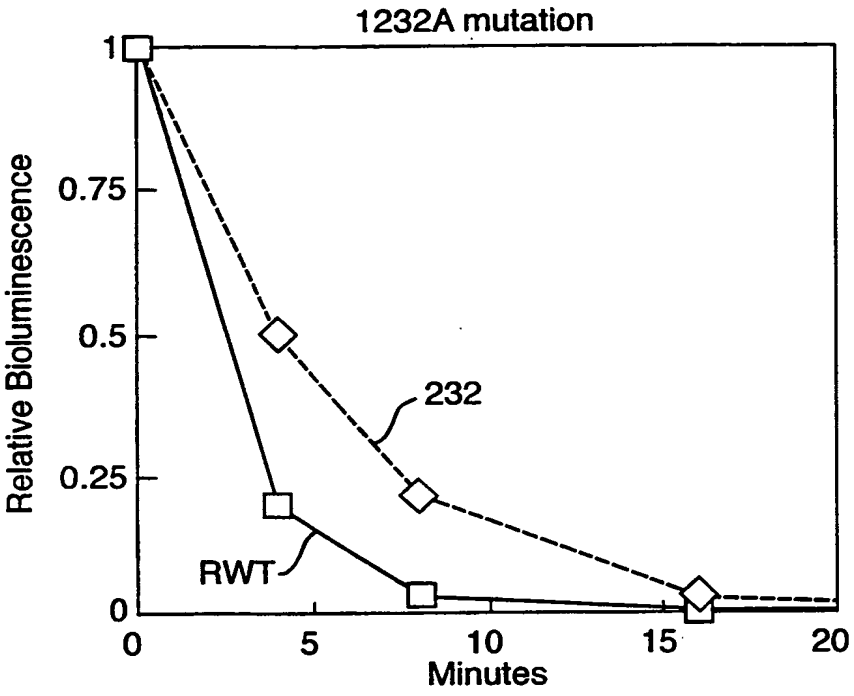
1232A mutation
Relative Bioluminescence
1
0.75
0.5
0.25
0
232
RWT
0
5
10
15
20
Minutes

Fig.3f.

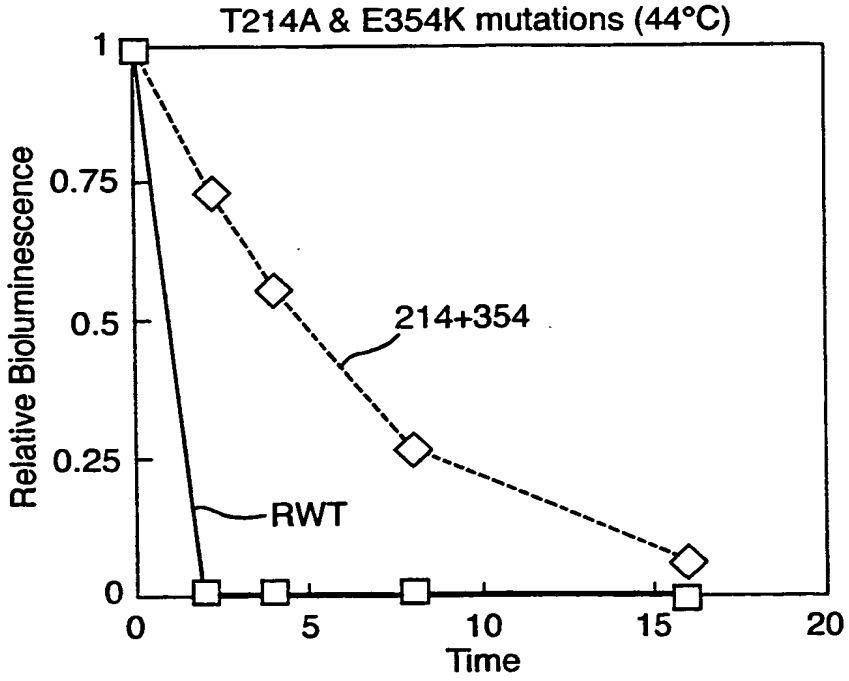
T214A & E354K mutations (44°C)
Relative Bioluminescence
1
0.75
0.5
0.25
0
214+354
RWT
0
5
10
15
20
Time

Fig.3g.

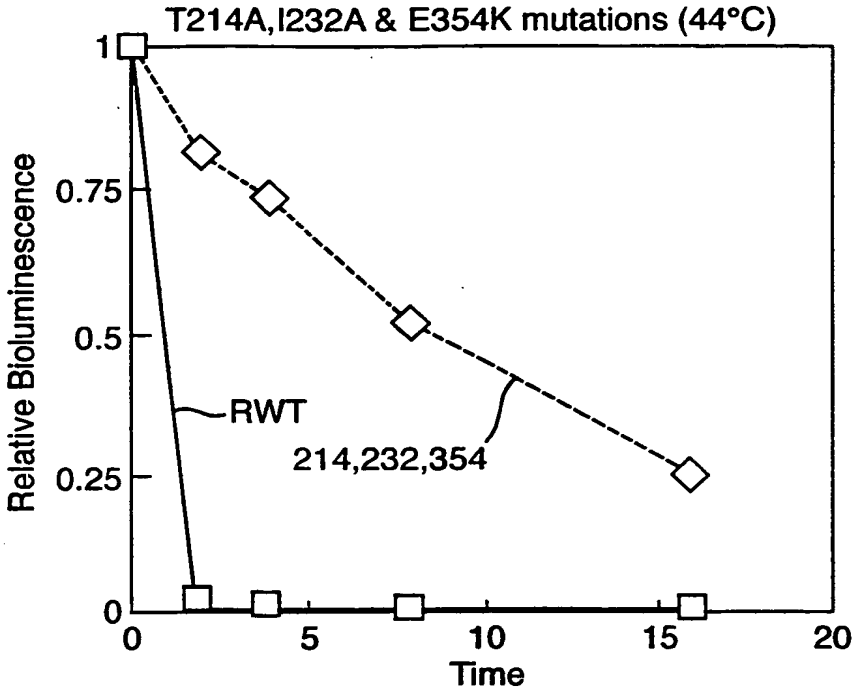
T214A,I232A & E354K mutations (44°C)
Relative Bioluminescence
1
0.75
0.5
0.25
0
RWT
214,232,354
0
5
10
15
20
Time

Fig.3h.

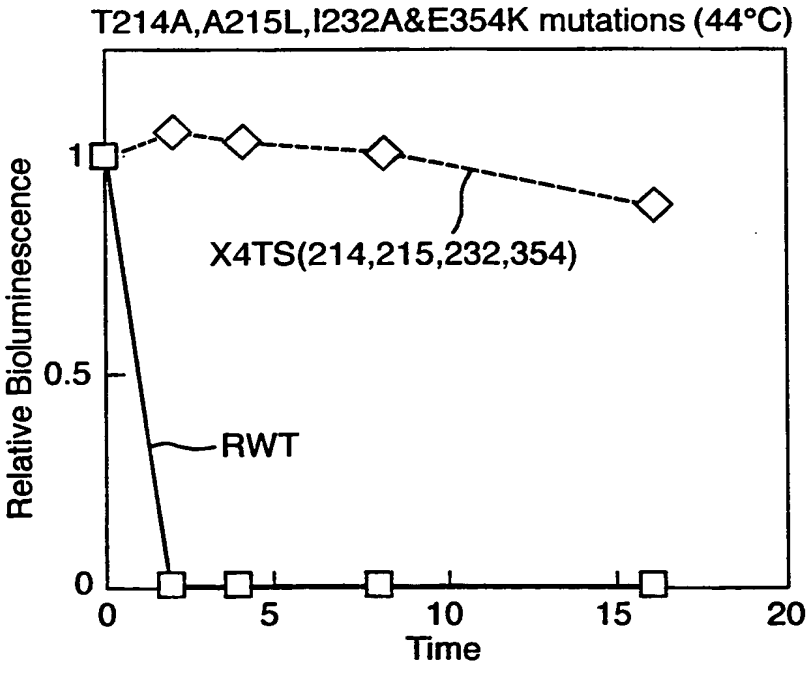
T214A,A215L,I232A&E354K mutations (44°C)
Relative Bioluminescence
1
0.5
0
X4TS(214,215,232,354)
RWT
0
5
10
15
20
Time

Fig.8.

CGCCGGTGAGCTCCCCGCCGCCG SACI-SENSE / 6371
CGGCGGCGGGGAGCTCACCGGCG SACI-ANTI / 6372
CGAACACTTCTTCATCGTTGACCGCCTTAAGTCTTTAATTAAATACAAAGG AFLII-SENSE / 6373
CCTTTGTATTTAATTAAAGACTTAAGGCGGTCAACTATGAAGAAGTGTTCG AFLII-ANTI / 6374
GAAAGGCCCGGCACCAGCCTATCCTCTAGAGG F14A-SENSE / 6375
CCTCTAGCGGATAGGCTGGTGCCGGGCCTTTC F14A-ANTI / 6376
CCATAAATTTACCGAATTCGTCGACTTCGATCGAGG C-TERM.SEQ/ 6641
GTGTGGAATTGTGAGCGG N-TERM.SEQ / 6651
GAGATACGCCGCGGTTCCTGG L35A-SENSE / 6652
CCAGGAACCGCGGCGTATCTC L35A-SENSE / 6653

CCCTATTTTCATTCCTGGCCAAAAGCACTG F295L-SENSE/ 9048
GAGTGCTTTTGGCCAGGAATGAAAATAGGG F295L-ANTI / 9049
CCGCATAGAgCTCTCTGCGTCAGATTC T214A + A215L-SENSE / 9063
GAATCTGACGCAGAGAGcTCTATGCGG T214A + A215L-ANTI / 9064
GTTGACCGCTTGGGATCCTTAATTAAATAC Insertion of BamHI at G339 / 9077

GTATAGATTTGAAAAAGAGCTG E270K-SENSE / 257
CAGCTCTTTTTCAAATCTATAC E270K-ANTI / 258
GGCTACATACTGGAGACATAGC S420T-SENSE / 629
GCTATGTCTCCAGTATGTAGCC S420T-ANTI / 630
GCAGTTGCGCCCGTGAACGAC A105L-SENSE / 790
GTCGTTCACGGGCGCAACTGC A105L-ANTI /791
CAAATCATTCCGGGTACTGCGATTTTAAG D234G-SENSE / 792
CTTAAAATCGCAGTACCCGGAATGATTTG D234G-ANTI / 793

CCGCATAGAACTCTCTGCGTCAGATTC A215L-SENSE / 7726
GAATCTGACGCAGAGAGTTCTATGCGC A215L-ANTI / 7727
CTGATTACACCCAAGGGGGATG E354K-SENSE / 7792
CATCCCCCTTGGGTGTAATCAG E354K-ANTI / 7793
ccettccgcatagannngcctgcgtcagt T214N-Sense / 8202
actgacgcaggcNNNtctatgcggaaggg T214N-Anti / 82033

GCAATCAAATCGCTCCGGATACTGC I232A-SENSE / 6911
GCAGTATCCGGAGCGATTTGATTGC I232A-ANTI / 6912

CCATTCCATCAAGGTTTTGG H245Q-SENSE / 9128

CCAAAACCTTGATGGAATGG H245Q-ANTI / 9129